Research Article
J. Food Sci. Technol. Nepal, Vol. 14 (7-18), 2024
ISSN: 1816-0727

Utilization of Psyllium Husk for Development of Synbiotic Soy Yoghurt and Cow Milk Yoghurt

Ranju Parajuli[1*], Tika Bahadur Karki[1], Roman Karki[2]

[1] *Department of Food Technology, National College of Food Science and Technology, Tribhuvan University, Kathmandu, Nepal*

[2]*National Food Research Centre, Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal*

Corresponding author: ranjuparajuli219@gmail.com

ABSTRACT

A study was carried out incorporating fiber-rich psyllium husk into soy yoghurt to enhance its nutritional profile and functionality and compare it with that of cow milk yoghurt. Four different formulations of soy yoghurt and cow milk yoghurt each were made using psyllium husk as a prebiotic and ABT7 (mixed culture: *L. bulgaricus, S. thermophillus, B. animalis*) as probiotics, with the first batch of each serving as a control. The best formulation was chosen based on sensory attributes and the selected formulations were then physically and chemically analyzed for various parameters such as pH, titratable acidity, water syneresis, total soluble solid (TSS), total reducing sugar, total phenol content, total flavonoid content, total antioxidant activity, and microbial counts for 12 days with three days of time interval. The study found that synbiotic soy yoghurt specifically with 0.5% psyllium husk, had the highest nutritional value, high viability, phenolic, flavonoid, and anti-radical activity, with levels ranging from 8.15-8.32 log CFU/ml, 2.21-2.84%, 5.64-25.53%, and 62-64% respectively and are significant ($p<0.05$) at 5% level of significance when stored for 12 days. The study revealed that the prepared synbiotic yoghurts were not suitable for storage beyond 12 days even at refrigeration temperature. However, the study resulted in the production of the fibre riched synbiotic soy yoghurt with best organoleptic and sensory properties within the storage period.

Keywords
Functional food, Prebiotic, Probiotic, Psyllium husk, Synbiotic

INTRODUCTION

Owing to the worldwide shortage of food and environmental concerns (Sobczak *et al*., 2023), attempts have been made to find alternative sources of protein, especially plant-based and soy proteins are on the top based on their nutritional and economic significance (Bedani *et al.,* 2013) as well as better digestibility (He and Chen, 2013). Moreover, due to different health-related issues to animal milk such as lactose intolerance, and increasing vegetarianism most people are shifting towards plant-based milk (Davoodi *et.al.,* 2013).

Soy milk is the non-fermented aqueous extract of cooked whole soybeans that contains about the same proportion of protein as cow's milk (around 3.5%), 2% fat, 2.9% carbohydrate, and 0.5% ash (Raja *et al.*, 2014). Soymilk resembles dairy milk in composition, like milk, it can also be fermented by lactic acid bacteria to produce soy yoghurt. Soy yoghurt, also known as *soygurt or yufu,* is a popular alternative to traditional dairy-based yoghurts in Asian Countries like Japan and China. Moreover, it doesn't contain cholesterol and lactose and is low in saturated fat (0.2 g/L), making it suitable for people with cardiovascular diseases and lactose intolerance (Mishra *et al.,* 2019). Since soy yoghurt is a fermented product, it has higher digestibility, increased vitamin and mineral availability, and reduced anti-nutritional factors (Vashishth *et al*., 2021; Hou *et al.,* 2000; Rackis, 1974; Nepali, 2007). Furthermore, fermentation results in a transformation of isoflavones to aglycones (Chien *et al.,* 2006) and improves absorption of isoflavones and free amino acids (Lee *et al.*, 2013) resulting in better absorptivity, reduced beany flavor, and increased antioxidant activity (Favaro *et al*., 2001; Marazza *et al.,* 2012; Zhao and Shah, 2014), enhancing both taste and health benefits.

Moreover, the nutritional and functional value of fermented soy-based products could be enhanced with the incorporation of probiotics, prebiotics, or both, which are prized by consumers. Since, the oligosaccharides, such as raffinose and stachyose, present in soybeans are not digested by humans, they could be ideal prebiotics, necessary for the growth of probiotic cultures (Marazza *et al.,* 2013). Thus, soybean alone or with other added prebiotics results growth of added probiotic microorganisms such as *S. thermophilus*, *L. acidophilus*, and *Bifidobacterium* grow in a considerable number with non-significant amounts of non-digestible oligosaccharides, resulting in a healthier product with added value such as synbiotic soy yoghurt for consumers (Bimo Setiarto *et al*., 2021)

Synbiotic is a combination of probiotics and prebiotics that supply synergy effects and improve the "friendly flora" of the human intestine (Patel *et al*., 2014). Here, prebiotics are the food materials comprehended by fibers of natural origin that are not digested in the gastrointestinal tract and improve the health of the host by selectively supporting the development and activity of particular genera of microorganisms in the colon, mostly probiotics like lactobacilli and bifidobacteria" (Pandey *et al*., 2015; Patel *et al*., 2014). Similarly, probiotics are live microorganisms that when administered in adequate amounts confer a health benefit to the host such as prevention of cancer, treatment of irritable bowel-associated diarrhea, reduction of serum cholesterol, anti-hypertensive effects, and improvement of lactose metabolism (Nagpal *et al*., 2012; Saarela *et al*., 2000).

The synbiotic product must contain between 10^6 and 10^8 colony forming units per gram (CFU/g) for optimum therapeutic effects (Martinez *et al*., 2015). However, during food processing and storage, the viable number of probiotics decreases due to the lack of energy sources of prebiotics making probiotics less tolerant to oxygen, low pH, and temperature. Without sufficient probiotics, digestion, absorption, and manufacture of nutrients in the human body cannot take place (Verma and Mogra, 2013). Therefore, the addition of psyllium husk, which is also a rich source of dietary fiber, not only serves as prebiotics in low-fat soya yoghurt but also improves their physicochemical and sensory properties by imparting fat-like textures and better gut health, and lowers calorie intake and blood cholesterol levels in humans (Bhat *et al.,* 2018 and Sierra *et al.,* 2001).

Soybean products offer many health benefits but its world consumption rate is low and faces many consumer objections due to off-flavors, flatulence from non-digestible oligosaccharides, and the presence of allergens and anti-nutritional factors. Therefore, developing synbiotic soy yogurt using locally available soybean and psyllium husk can address these issues by enhancing flavor, digestibility, and overall consumer acceptance. Considering all, this study aims to determine the optimum proportion of psyllium husk in the preparation of the synbiotic soy yoghurt with good sensorial acceptability and compare it with cow milk-based symbiotic yoghurts in terms of physicochemical and microbial stability.

MATERIALS AND METHODS

Prebiotic and Probiotic

Psyllium husk used as a prebiotic was purchased from Health for all Pvt. Ltd, Lalitpur, Nepal, and freeze-dried mixed probiotic culture (*L. bulgaricus*, *S. thermophillus*, *B. animalis*) of strain ABT7 was obtained from Dairy Development Corporation (DDC) in sealed packages.

Preparation of Soymilk

Soymilk was prepared as per Nsofor and Maduako (1992) using white soybean variety. Soybean, soaked in 5% $NaHCO_3$ solution for 15 hours, was decapitated and subsequently washed with water. It was then ground along with warm water (1:7 ratio) in a chopper mixer grinder-550W (KONKA, Shenzhen, China), and was filtered through muslin cloth. It was then heated at 90°C for 30 minutes and was filtered to remove okra (solid residue) and the soymilk was obtained.

Mixing of psyllium husk and sugar to soymilk and cow milk

To the previously pasteurized and cooled soymilk and cow milk, different proportions (0%, 0.3%, 0.5%, or 0.7% (w/v) of prebiotic (psyllium husk) were added in respective vials as per Bhat *et.al* (2018) with some modifications. The sucrose (8% w/v) was added to each formulation to enhance palatability. It was then stirred until mixing properly without letting the temperature of the mixture fall below 37°C. The freeze-dried probiotic culture ABT7 powder was added at the rate of 0.02% (w/v), stirred thoroughly, and incubated at 37°C for 6 hours until the pH value reached close to 5 for soy yoghurt (Raksalam, 2018) and for 4 hours until pH value reached close to 4.4 for cow milk yoghurt (Vargas *et al.,* 2008). Eight different synbiotic soy yoghurt and cow milk yoghurts were prepared (Table 1), and their below-mentioned properties were determined.

Determination of Physico-chemical parameters

The moisture content, crude protein, crude fat, crude fiber, ash, and total carbohydrate of raw soybean, psyllium husk, and different yoghurt samples were determined as per AOAC (2005).

Table 1: Different formulated synbiotic yoghurt with their sample code

Formulation	Sample code
Control soy yoghurt	CS
Soy yoghurt with 0.3% psyllium husk	S1
Soy yoghurt with 0.5% psyllium husk	S2
Soy yoghurt with 0.7% psyllium husk	S3
Control cow milk yoghurt	CM
Cow milk yoghurt with 0.3% psyllium husk	M1
Cow milk yoghurt with 0.5% psyllium husk	M2
Cow milk yoghurt with 0.7% psyllium husk	M3

pH and Titratable acidity

pH measurements were carried out at room temperature (27°C) using a digital pH211 microprocessor pH meter (Hanna Instruments ®, Nusfalau, Romania) (AOAC, 2005) and that of titratable acidity was measured by titration with 0.1N NAOH solutions and using 1% phenolphthalein as an indicator as per AOAC (2005). Both the pH and titratable acidity were measured for 12 days at a three-day interval.

Total soluble solids (TSS)

TSS was determined by using a Model: FG 103 portable refractometer, (Kesari Scientific Chemicals, Chennai, India) (AOAC, 2005) in terms of degree Brix for all soy and cow milk yoghurt samples. It was also measured until the twelfth day at a three-day interval.

Water syneresis

Syneresis was measured using the method described by Mei *et al*. (2017) with slight modifications. Twenty grams of each yoghurt sample were placed in a funnel lined with a Whatmann filter paper (no. 1), which was then maintained at 4°C for 5 h, and the obtained liquid volume was recorded. Syneresis was calculated using the following equation:

$$\text{Syneresis (\%)} = (V_1/V_2) \times 100$$

Where,

V_1 denotes the volume of whey collected after drainage and

V_2 denotes the volume of the yoghurt sample

Determination of total phenol and total flavonoid content

Total phenols were determined with the Folin-Ciocalteau reagent (FC-reagent) as per Makkar *et al.* (1993) and expressed as mg of Gallic acid equivalent (GAE) of phenol/100g of dry sample whereas the total flavonoids were determined using UV spectrophotometer where Quercetin was used as reference standard as per Chang *et al.* (2002) and expressed as µg qE/mg extract.

Determination of Antioxidant Activity

The total antioxidant activity by the DPPH radical scavenging method was determined according to the method described by Brand-Williams *et al.* (1995) for 12 days at a three-day interval. The corresponding percentage of DPPH inhibition was then calculated at an absorbance of 517 nm using a UV1800 120V UV/Vis Spectrophotometer (Shimadzu, Kyoto, Japan. The total AA was calculated as radical scavenging activity (%RSA) by using the formula:

$$\text{\% Radical scavenging activity} = [(Ac-As)/Ac] \times 100$$

Where, Ac= absorbance of the control (1 ml Methanol + 2 ml DPPH) and As= absorbance of the sample.

Determination of total viable probiotic count

The total viable probiotic count was determined by using the Standard Plate Count (SPC) method as described by Shori *et al.,* 2022. De Man, Rogosa, and Sharpe (MRS) agar media containing 1% calcium carbonate were used for the propagation of the lactobacilli strains present in the samples. After incubation, the inoculated plates having 30-300 colonies were considered for counting and expressed as log CFU/ml of probiotic bacteria in the synbiotic yoghurts.

Determination of yeast and mold count

Yeast and mold count were determined as per Tournas *et al.* (2001) using Potato Dextrose Agar (PDA) as a medium. The inoculated plates were counted for colonies and expressed as log CFU/ml of yeast and mold in the synbiotic yoghurt.

Sensory Evaluation

Sensory evaluation was done with ten semi-trained panelists using 9-point hedonic scoring scale (9 = "like extremely" and 1 = "dislike extremely") for the six parameters of color, flavor, appearance, texture, sourness, and overall acceptance of all coded samples as described by (Ranganna, 1986).

Research Article
J. Food Sci. Technol. Nepal, Vol. 14 (7-18), 2024
ISSN: 1816-0727

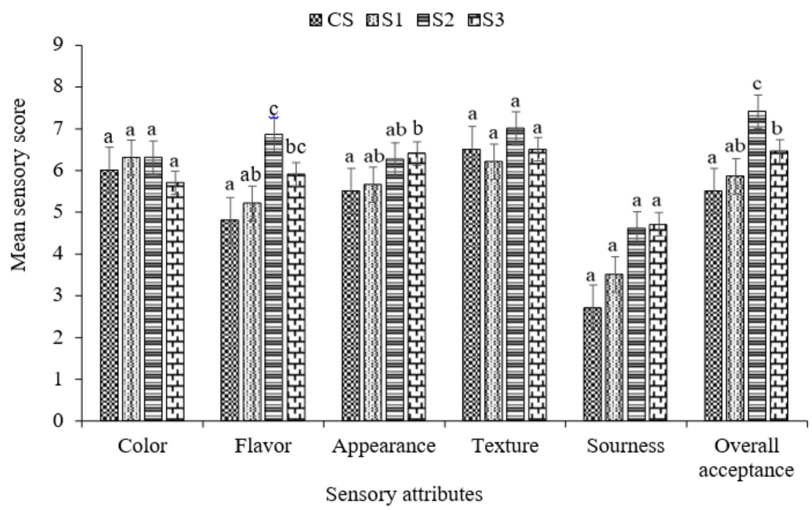
Data analysis

The determined parameters of the triplicates of all samples were statistically analyzed using SPSS (IBM Version 26.0: IBM Corp, USA.). The proximate composition of the best samples (S2 and M2) along with their controls (CS and CM) were analyzed by one-way ANOVA and a post hoc test was done using the Tukey test for significant factors. Similarly changes in pH, total acidity, TSS, Water syneresis, total antioxidant activity, and total viable count from day 1 to 12 for the samples with different proportions of psyllium husk in soy or cow milk yoghurt were analyzed by two-way analysis of variance (ANOVA) using a full factorial model (IBM SPSS Statistics for Windows, Version 26.0: IBM Corp, USA). The posthoc test, when there was significant interaction was analyzed by LSD tests for multiple comparisons with the adjusted level of significance (Bonferroni correction) of α/ no of comparisons (0.005% for storage days and 0.0083% for different sample formulations

RESULTS AND DISCUSSION

Sensory evaluation of the sample

The samples with 0.5% psyllium husk incorporation (S2, M2) were found to be best with scores of approx. 7.4 and 8 respectively. (Figure 1). There is variation in the overall acceptance among yogurt samples because as the proportion of psyllium husk increases, the texture, appearance, flavor and overall acceptance also increased but to the husk proportion of 0.5% only because with further increment it affects the organoleptic properties which could be further confirmed by samples CS and M3 which had lowest overall acceptance than that of samples S2 and M2. The sensory score based on overall acceptance of different prepared soy yoghurt and cow milk yoghurt, LSD ($p<0.05$), shows a significant difference between the different psyllium husk incorporated soy yoghurt samples and cow milk yoghurt samples. Therefore, those samples were accepted and further analysis (physicochemical, microbiological) of these two samples was done along with their comparison with control ones.



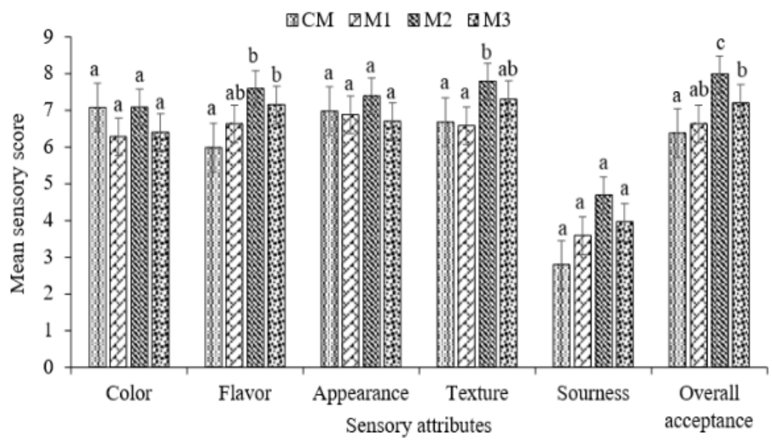


Figure 1 Sensory score for different attributes of soy and cow milk yoghurts
Note: CS- control soy yoghurt, S2- soy yoghurt with 0.5% psyllium husk,CM- control cow milk yoghurt, M2- cow milk yoghurt with 0.5% psyllium husk,
Values presented are means ± standard deviation (SD) of triplicate values.
[a–d] Values with different letters in the superscripts differ significantly (p<0.05)

Proximate composition of the best samples from sensory evaluation
From the data given in Table 1, the proximate composition of the product indicated that protein increased along with crude fiber content due to the incorporation of psyllium husk which is rich in fiber, and that also due to fermentation (Bhat *et al.*, 2018). On the contrary, the fat and total carbohydrate contents decreased most likely due to their utilization by the growing probiotic microorganisms (Yeo and Liong 2010).

Changes in different parameters of the best samples based on sensory evaluation
Change in pH
The change in pH and acidity were determined after 1 day, 3 days, 6 days, 9 days, and 12 days of fermentation. There was a significant effect (p <0.05) of storage time (days) and formulations on the pH without a significant (p >0.05) interaction term. The pH decreased significantly after 9 or 12 days of storage compared to 3 days of storage showing a decreasing trend with the increase in fermentation time while acidity shows an increasing progression. (Table 2)

For all the samples, pH decreased statistically with increasing storage time but was significantly lower in psyllium husk-treated samples over control ones i.e. CS (4.76-4.52), CM (4.62-4.48) and S2 (4.66-4.42), M2 (4.64-4.4). Adding psyllium husk seems to decrease pH in the case of both types of synbiotic yoghurts within the 12th day of refrigerated storage. The decreased pH throughout the storage period might be due to the formation of lactic acid by certain bacteria in yoghurt (Athar *et al.*, 2000).

Change in total acidity
Similarly, there was a significant effect (p <0.05) of storage time (days) and formulations on the pH but with a significant (p <0.05) interaction term, indicating that the effect of storage time on total acidity (% acetic acid) depends on the different formulation. it was seen that the percentage of total acidity goes on increasing with the fermentation time from CS(0.18-0.62)%, S2(0.23-0.71)%, CM(0.21-0.68)%, M2(0.22-0.69)%), but there was only the slightest change in acidity due to refrigeration storage which hindered acid production by LAB. Moreover, the total acidity of the psyllium husk added sample showed a higher acidity than that of the control synbiotic yoghurts, which may be due to the production of more lactic acid by LAB as psyllium husk appeared to increase the lactic acid percentage significantly acting as prebiotic (Mabrouk and Effat, 2020). Titratable acidity also depends on the amount of carbohydrate contained in the yoghurt samples, lactose content in cow milk is high thus cow based yoghurts showed high titratable acidity compared to soy yoghurts (Jimoh and Kolapo, 2007).

Change in TSS
There was a significant effect (p <0.05) of storage time (days) and formulations on the TSS levels without a significant (p >0.05) interaction term. The yoghurt with psyllium husk had a

significantly higher (p <0.05) TSS than the control which may be due to the addition of soluble dietary fiber (psyllium husk) (reference). With an increase in storage time, TSS decreased significantly (p <0.05), which may be due to the metabolism of sugars by probiotic mixed culture (Soni *et al.,* 2020) The cow milk-based synbiotic yoghurt with psyllium husk addition as prebiotic (M2) has the highest value of TSS (12.07°Bx) on day 1 which goes on decreasing and reaches 11.86°Bx in day 12. Also, cow milk yoghurt had higher solid content than soy yoghurt which may be due to the presence of lactose in cow milk and other carbohydrates which is absent or present in considerably fewer amounts in soy milk (Karki *et al.,* 2014).

Change in water syneresis

There was also a significant effect (p <0.05) of storage time (days) and formulations on the water syneresis but with a significant (p >0.05) interaction term. The water syneresis significantly increased (p <0.05) with an increase in storage time from 1 to 12 days. The sample CS had higher water syneresis as compared to S2 (40.9%) and that of sample CM (47.1%) was higher than that of M2 (46.21%). The higher value in the plain yoghurts could, therefore, be inferred that the addition of psyllium husk improves the structure of the yoghurt samples, which prevents unwanted syneresis, due to the physicochemical properties of the husk which implies that free water molecules within the yoghurt matrix were better absorbed with increasing psyllium husk proportion (Cais-Sokolinska *et al.*, 2002). Furthermore, plant-based yoghurts such as soy yoghurt contain higher hydrocolloids resulting in higher WHC compared to of dairy yoghurts increasing gel firmness and preventing serum separation for extended shelf life (Grasso *et al.,* 2020).

Change in total phenol content

There was a significant interaction of storage period on the total phenol content, p<0.05. Similarly, there was a significant effect of formulations, p<0.0083 except for formulation CS and M2, p>0.0083, which shows no significant difference on day 12 of the storage period and that of sample CS and M2 on day 1 and that of the interaction term storage days-formulations was also significant, p<0.05 (Table 3). The observed values for soy yoghurt samples were slightly lower but comparable range as reported by Yang *et al.* (2013a) which is 2.824 mg GAE 100 g which is 2.5±0.04 mg GAE/g for cow milk yoghurt as per Arampath *et al.* (2021). There was an increase in total phenolic content with the increase in storage period because of the proteolytic activity of yogurt bacteria which release some phenolic compounds such as phenolic acids, flavonoids, and iso-flavonoids present in milk and soymilk respectively (Ainsworth and Gillespie, 2007).

Change in total flavonoid content

From the statistical analysis, the total flavonoid content of different formulations was found to have significant storage days, p<0.005, formulations, p<0.0083, and time-temperature interaction, p<0.05 (Table 3). It was found that the soy milk and cow milk yoghurts with psyllium husk had the highest flavonoid content than that without husk i.e. CS (19.63 mg/ml), S2 (25.53 mg/ml), CM (11.43 mg/ml) and M2 (20.24 mg/ml) which may be due to the release of phenolic compounds presence in the psyllium husk which got added to that of soymilk and cow milk phenolic compounds.

Change in total antioxidant activity

The DPPH radical scavenging activity was highest for synbiotic yoghurt samples as compared to control ones and among all the synbiotic soy yoghurt samples had the highest i.e. S2(62.18%) followed by M2 (46.06%). It was revealed that both storage days and formulation have a significant effect on antioxidant activity, with p< 0.05 and p<0.0083 respectively except for the sample S2 and M2 on day 1 which shows no significant difference at p>0.0083. In addition, the results show a significant interaction effect between storage days and formulation, p<0.05 (Table 3). Our observed values were in the range reported by Pasqualetti *et al.* (2014) and Silalahi *et al.* (2018) and higher than the values reported by Yang *et al.* (2013b) which is 28.39%. There is a rapid decrease in DPPH% in the case of cow milk-based yoghurt i.e. CM (63.62 to 42.77%) and M2 (64.50 to 46.06%) which may be due to the hydrolysis of milk and soy protein or organic acid production by microorganism during fermentation and storage under refrigeration (Cho *et al.*, 2020).

Research Article
J. Food Sci. Technol. Nepal, Vol. 14 (7-18), 2024
ISSN: 1816-0727

Table 1: Chemical composition of different yoghurts

Parameters	Formulations			
	CS	S2	CM	M2
Moisture (%)	90.70 ± 0.25^{c}	91.42 ± 0.11^{d}	88.55 ± 0.07^{a}	89.84 ± 0.10^{b}
Crude protein (%db)	24.37 ± 0.21^{a}	30.68 ± 0.17^{b}	30.55 ± 0.08^{b}	37.35 ± 0.0^{c}
Crude fat (%db)	13.95 ± 0.16^{a}	16.80 ± 0.11^{b}	20.10 ± 0.17^{c}	23.91 ± 0.09^{d}
Crude fiber (%db)	0.64 ± 0.10^{b}	1.84 ± 0.11^{d}	0.17 ± 0.08^{a}	1.08 ± 0.09^{c}
Total ash (%db)	3.99 ± 0.10^{c}	5.71 ± 0.17^{d}	2.04 ± 0.18^{a}	3.55 ± 0.09^{b}
Net Carbohydrate (%db)	57.28 ± 0.75^{d}	43.68 ± 0.89^{b}	47.61 ± 0.79^{c}	34.41 ± 0.68^{a}

Where,
CS- control soy yoghurt, S2- soy yoghurt with 0.5% psyllium husk,CM- control cow milk yoghurt, M2- cow milk yoghurt with 0.5% psyllium husk,
Values presented are means ± standard deviation (SD) of triplicate values.
$^{a–d}$ Values with different letters in the superscripts differ significantly ($p<0.05$)

Table 2: Physicochemical analyses of different yoghurt samples

	Storage days	Formulations					Parameters	Storage days	Formulations	Storage days* Formulations
		CS	S2	CM	M2	**Overall**	Df	(4,40)	(3,40)	(12,40)
	1	4.76±0.07^{a}	4.66±0.05^{a}	4.62±0.09	4.64±0.08^{a}	4.67±0.08^{c}	F Value	12.963	5.606	0.269
	3	4.69±0.09^{a}	4.6±0.08^{a}	4.57±0.06	4.55±0.07^{a}	4.6±0.08^{bc}	p Value	<0.005	0.003	0.991
pH	6	4.62±0.12^{a}	4.54±0.12^{a}	4.53±0.07	4.48±0.05^{a}	4.54±0.09^{ab}	Partial η2	1.0	0.565	0.296
	9	4.53±0.09^{b}	4.47±0.07^{a}	4.49±0.1	4.46±0.04^{a}	4.49±0.07^{a}				
	12	4.52±0.06^{b}	4.42±0.08^{b}	4.48±0.1	4.4±0.08^{b}	4.45±0.08^{a}				
	Overall	**4.62±0.12^{X}**	**4.53±0.1^{W}**	**4.54±0.09^{W}**	**4.54±0.09^{W}**					
	1	0.21±0.004^{aW}	0.18±0.002^{aX}	0.27±0.001^{aY}	0.23±0.001^{aZ}	0.22±0.03	F Value	45098.4	2644.46	913.04
	3	0.27±0.002^{bW}	0.25±0.02^{bX}	0.38±0.001^{bY}	0.29±0.001^{bZ}	0.30±0.05	p Value	<0.005	<0.0083	<0.05
Acidity	6	0.4±0.006^{cW}	0.47±0.002^{cX}	0.47±0.00^{cX}	0.48±0.002^{cZ}	0.45±0.03	Partial η2	1.0	0.995	0.996
	9	0.48±0.003^{dW}	0.56±0.001^{dX}	0.51±0.003^{dY}	0.58±0.001^{dZ}	0.53±0.04				
	12	0.52±0.002^{eW}	0.65±0.001^{eX}	0.59±0.002^{eY}	0.71±0.002^{eZ}	0.62±0.07				
	Overall	**0.38±0.12**	**0.42±0.18**	**0.44±0.11**	**0.46±0.185**					
	1	10.21±0.01^{aW}	11.24±0.00^{aY}	10.49±0.17^{aX}	12.07±0.06^{aZ}	11.0±0.75^{e}	F Value	100.531	13658.473	1.920
	3	10.16±0.00^{bW}	11.22±0.00^{bY}	10.46±0.02^{bX}	11.99±0.02^{bZ}	10.96±0.74^{d}	p Value	<0.005	<0.0083	0.061
TSS	6	10.14±0.00^{cW}	11.15±0.02^{cY}	10.44±0.00^{cX}	11.95±0.01^{bZ}	10.92±0.72^{c}	Partial η2	0.91	0.99	0.365
	9	10.06±0.02^{dW}	11.07±0.06^{dY}	10.40±0.0^{dX}	11.90±0.01^{cZ}	10.85±0.73^{b}				
	12	10.01±0.01^{dW}	11.02±0.03^{dY}	10.34±0.04^{dX}	11.86±0.02^{dZ}	10.81±0.73^{a}				
	Overall	**10.11±0.07^{W}**	**11.14±0.09^{Y}**	**10.43±0.05^{X}**	**11.95±0.08^{Z}**					
	1	44.5±0.12^{aW}	40.9±0.04^{aX}	47.1±0.15^{aY}	46.21±0.11^{aZ}	44.6±2.48	F Value	2536.57	14294.81	81.47
	3	44.9±0.04^{bW}	41.3±0.02^{bX}	47.5±0.01^{bY}	46.62±0.16^{bZ}	45.08±2.48	p Value	<0.005	<0.0083	<0.05
Water syneresis	6	45.6±0.08^{cW}	41.8±0.1^{cX}	48.2±0.09^{cY}	47.1±0.08^{cZ}	45.68±2.53	Partial η2	0.996	0.999	0.961
	9	45.9±0.11^{dW}	42.4±0.01^{dX}	48.9±0.1d^{Y}	47.81±0.01^{dZ}	46.25±2.58				
	12	46.5±0.03^{eW}	45.2±0.13^{eX}	50.12±0.04^{eY}	49.86±0.03^{eZ}	47.92±2.21				
	Overall	**45.48±0.73**	**42.32±1.57**	**48.36±1.1**	**47.52±1.33**					

Values presented are means ± SD of triplicate values.

Values without the superscripts do not differ significantly (p>0.05).

Where, CS- control soy yoghurt, S2- soy yoghurt with 0.5% psyllium husk, CM- control cow milk yoghurt, M2- cow milk yoghurt with 0.5% psyllium husk

$^{a–e}$ Values with different letters within in the same column differ significantly (p<0.05)

$^{W–Z}$ Values with different letters within the same row differ significantly (p<0.05)

J. Food Sci. Technol. Nepal, Vol. 14 (7-18), 2024
ISSN: 1816-0727

Table 3: Analysis of Bioactive components of different yoghurt samples

	Storage days	Formulations					Parameters	Storage days	Formulations	Storage days* Formulations
		CS	S2	CM	M2	**Overall**	df	(1,16)	(3,16)	(3,16)
Total antioxidant activity	1	62.74±0.04^{aY}	64.49±0.0^{aX}	63.62±0.01^{aW}	64.5±0.01^{aX}	63.84±0.7	F Value	915936.19	139130.42	141567.46
	12	57.3±0.00^{bY}	62.18±0.0^{bZ}	42.77±0.05^{bW}	46.06±0.04^{bX}	52.08±8.2	p Value	<0.05	<0.0083	<0.05
	Overall	**60.02±2.97**	**63.33±1.2**	**53.19±11.4**	**55.28±10.09**		Partial η2	1.0	1.0	1.0
Total phenol content	1	2.52±0.02^{aX}	2.84±0.01^{aY}	2.01±0.02^{aW}	2.43±0.02^{aX}	2.45±0.31	F Value	895.55	402.44	16.89
	12	2.02±0.06^{bX}	2.21±0.02^{bY}	1.45±0.07^{bW}	2.12±0.02^{bXY}	1.95±0.31	p Value	<0.05	<0.0083	<0.05
	Overall	**2.27±0.27**	**2.52±0.34**	**1.73±0.31**	**2.27±0.17**		Partial η2	0.982	0.987	0.76
Total flavonoid content	1	19.63±0.02^{aX}	25.53±0.03^{aZ}	11.43±0.03^{aW}	20.24±0.03^{aY}	19.21±5.26	F Value	1175330.12	65097.94	26906.71
	12	4.07±0.03^{bX}	5.64±0.04^{bZ}	2.54±0.04^{bW}	4.33±0.03^{bY}	4.14±1.15	p Value	<0.05	<0.0083	<0.05
	Overall	**11.85±8.51**	**15.58±10.89**	**6.98±4.87**	**12.28±8.7**		Partial η2	1.0	1.0	1.0

Where, CS- control soy yoghurt, S2- soy yoghurt with 0.5% psyllium husk, CM- control cow milk yoghurt, M2- cow milk yoghurt with 0.5% psyllium husk
Values presented are means ± SD of triplicate values.
Values without the superscripts do not differ significantly ($p > 0.05$).
$^{a–c}$ Values with different letters within in the same column differ significantly ($p < 0.05$)
$^{W–Z}$ Values with different letters within the same row differ significantly ($p < 0.05$)

Change in total viable count

There was a significant difference between the storage days, p<0.0083 and that of formulation was also significant, p<0.0083. Similarly, a significant interaction effect between storage days and formulation, p<0.05 was observed. The probiotic count of the yoghurt samples decreased with the storage period which may be due to the low survivability rate of the probiotics as the availability of prebiotics goes on decreasing with the fermentation period. It was found that the sample with prebiotics (psyllium husk) showed better retention of viability (8.0 log CFU/ml) till the storage period of 12 days i.e. S2 (8.32±0.15) and M2 (8.63±0.02) in comparison to that of the yoghurts without prebiotics which may be due to the presence of prebiotics in synbiotic yoghurt which enhanced the viability of the probiotic strain present in it because of their ability to be fermented by the lactobacilli strains.

Mold count

The mold count of different prepared yoghurt samples were calculated on subsequent refrigerated storage period i.e. day 1, day 4, day 8 and day 12 and no any molds were not detected in any of the samples within the 12 days of storage which ensures the good microbial quality of the prepared yoghurts.

CONCLUSION

From the study, it can be found that adding psyllium husk (0.5%) to yoghurt samples improved both sensory and nutritional attributes along with enhanced probiotic viability. Hence, probiotics and prebiotics can be used in appropriate combinations to make synbiotic products with added health benefits due to the presence of a high concentration of iso-flavones, which was evident from higher anti-radical activity. This highlights its potential for addressing dietary needs like lactose intolerance and protein deficiency and provides insights for developing functional dairy products promoting digestive health.

REFERENCES

Agrawal, R. (2021). Psyllium: A Source of Dietary Fiber. In: "Dietary Fibers". (Waisundara, V.Y., Eds). IntechOpen Limited Publishers. London.

AOAC. (2005). "Official methods of analysis" (18th ed.). Association of Official Analytical Chemists. Washington DC.

Athar, I. H., Shah, M. A. and Khan, U. (2000). Effect of various stabilizers on whey separation (syneresis) and quality of yoghurt. Pak. J. Biol. Sci. (Pakistan)

Bedani, R., Rossi, E. A. and Saad, S. M. I. (2013). Impact of inulin and okara on Lactobacillus acidophilus La-5 and Bifidobacterium animalis Bb-12 viability in a fermented soy product and probiotic survival under in vitro simulated gastrointestinal conditions. *J. Food Microbiol.* **34** (2), 382-389

Bhat, S. V., Deva, A. M. and Amin, T. (2018). Physicochemical and textural properties of yogurt fortified with psyllium (Plantago ovate) husk. *J. Food Process. Preserv*, **42** (2), e13425.

Bimo Setiarto, R. H., Widhyastuti, N. and Kurnia, D. R. (2021). Optimal concentration of prebiotic raffinose to increase viability of Lactobacillus acidophilus, Lactobacillus bulgaricus, Streptococcus thermophilus. *Carpathian J. Food Sci. Technol.* **13** (3).

Blackwood, A. D., Salter, J., Dettmar, P. W. and Chaplin, M. F. (2000). Dietary fibre, physicochemical properties and their relationship to health. *J. R. Soc. Promot. Health*, **120** (4), 242-247.

Blumenthal, M., Goldberg, A. and Brinckmann, J. (2000). *"Herbal medicine. Expanded commission E monographs"*. Integrative Medicine Communications Publishing. Newton, USA.

Brand-Williams, W., Cuvelier, M.-E. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT- J. Food Sci. Technol.* **28** (1), 25-30.

Cais-Sokolińska, D., Pikul, J. and Danków, R. (2002). Quality and stability of the natural yogurt produced from milk condensed with whey protein concentrate. *J. Sci. Technol.* **5** (2).

Chang, C.C., Yang, M.H., Wen, H.M. and Chern, J.C., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal. 10*(3).

Chien, H.L., Huang, H.Y., & Chou, C.C. (2006). Transformation of isoflavone phytoestrogens during the fermentation of soymilk with lactic acid bacteria and bifidobacteria. *J. Food Microbiol, 23*(8), 772-778.

Cho, W. Y., Kim, D. H., Lee, H. J., Yeon, S.-J. and Lee, C. H. (2020). Quality characteristic and antioxidant activity of yogurt containing olive leaf hot water extract. *CyTA- J. Food*, **18** (1), 43-50.

Davoodi, H., Esmaeili, S., & Mortazavian, A. (2013). Effects of milk and milk products consumption on cancer: a review. *Comprehensive Reviews in Food Science and Food Safety, 12*(3), 249-264.

Favaro , C., Terzi, S., Trugo, L., Della Modesta, R. and Couri, S. (2001). Development and sensory evaluation of soy milk based yoghurt. *Archivos latinoamericanos de nutrición*, **51** (1), 100-104.

Grasso, N., Alonso-Miravalles, L., & O'Mahony, J. A. (2020). Composition, physicochemical and sensorial properties of commercial plant-based yogurts. *Foods,* **9** (3), 252.

He, F.J. and Chen, J.Q. (2013). Consumption of soybean, soy foods, soy isoflavones, and breast cancer incidence: Differences between Chinese women and women in Western countries and possible mechanisms. **2** (3-4), 146-161.

Hou, J.W., Yu, R.C. and Chou, C.C. (2000). Changes in some components of soymilk during fermentation with bifidobacteria. *J. Food Res. Int.* **33** (5), 393-397.

Jimoh, K. and Kolapo, A. (2007). Effect of different stabilizers on acceptability and shelf stability of soy yoghurt. *Afr. J. Biotechnol.* **6** (8).

Karki, T., Shrestha, S., Bohara, B. and Jyakhwa, U. (2014). Characterization and Comparison of Soy Milk and Buffalo Milk Based Synbiotic Product. *J. Food Sci. Technol. Nepal*, **8**. 10.3126/jfstn.v8i0.11737.

Kumar Mishra, B., Hati, S., Das, S., & Prajapati, J. B. (2019). Biofunctional attributes and storage study of soy milk fermented by Lactobacillus rhamnosus and Lactobacillus helveticus. *J. Food Technol. Biotechnol., 57*(3), 399-407.

Mabrouk, A., & Effat, B. (2020). Production of high nutritional set yoghurt fortified with quinoa flour and probiotics. *Intl. J. Environ. Agric. Biotechnol., 5*(6), 1529-1537.

Makkar, H. P., Blümmel, M., Borowy, N. K. and Becker, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *J. Sci. Food.Agri.* **61** (2), 161-165.

Marazza, J. A., Nazareno, M. A., de Giori, G. S. and Garro, M. S. (2012). Enhancement of the antioxidant capacity of soymilk by fermentation with Lactobacillus rhamnosus. *J. Funct. Foods*. **4** (3), 594-601.

Martinez, R. C. R., Bedani, R. and Saad, S. M. I. (2015). Scientific evidence for health effects attributed to the consumption of probiotics and prebiotics: an update for current perspectives and future challenges. **114** (12), 1993-2015.

Mei, J., Feng, F. and Li, Y. (2017). Effective of different homogeneous methods on physicochemical, textural, and sensory characteristics of soybean (Glycine max L.) yogurt. *Taylor & Francis*. **15** (1), 21-26. 10.1080/19476337.2016.1197315.

Nepali, S. K. (2007). Comparative study of the kinema prepared from three varieties of soybeans using pure culture of Bacillus subtilis. Unpublished B.Tech Thesis.Tribhuvan Univ., Nepal.

Nsofor, L. M., & Maduako, O. (1992). Stabilized soymilk for ambient tropical storage: A preliminary report. *Intl. J. Food Sci. Technol, 27*(5), 573-576.

Pasqualetti, V., Altomare, A., Guarino, M. P. L., Locato, V., Cocca, S., Cimini, S., Palma, R., Alloni, R., De Gara, L., Cicala, M. (2014). Antioxidant activity of inulin and its role in the prevention of human colonic muscle cell impairment induced by lipopolysaccharide mucosal exposure. *PLoS One*, *9*(5), e98031.

Patel, P. J., Singh, S. K., Panaich, S. and Cardozo, L. (2014). The aging gut and the role of prebiotics, probiotics, and synbiotics: A review. *J. Clin. Gerontol. Geriatr.* **5** (1), 3-6.

Rackis, J. J. (1974). Biological and physiological factors in soybeans. **51** (Part2), 161A-174A.

Raja, J., Punoo, H. A. and Masoodi, F. A. (2014). Comparative study of soy paneer prepared from soymilk, blends of soymilk and skimmed milk. *J. Food Process. Technol*. **5** (301), 2.

Raksalam, M., Srisopha, W. and Sirimuangmoon, C. (2018). Development of soy yogurt drink fortified with quinoa and psyllium husk. **28** (2), 413-425.

Ranganna, S. (1986). "Handbook of analysis and quality control for fruit and vegetable products". Tata McGraw-Hill Education Publishing.

Shori, A. B., Albalawi, A., Al Zahrani, A. J., Al-sulbi, O. S. and Baba, A. S. (2022). Microbial analysis, antioxidant activity, and sensory properties of yoghurt with different starter cultures during storage. *Int. J. Dairy. Sci*. **126**, 105267.

Sierra, M., García, J. J., Fernández, N., Diez, M. J., Calle, A. and Sahagún, A. M. (2001). Effects of ispaghula husk and guar gum on postprandial glucose and insulin concentrations in healthy subjects. *Eur. J. Clin. Nutr.* **55** (4), 235-243.

Silalahi, J., Nadarason, D. and Silalahi, Y. (2018). The effect of storage condition on antioxidant activity of probiotics in yoghurt drinks. *Asian J. Pharm. Clin. Res.* **11**, 2455-3891.

Sobczak, P., Grochowicz, J., Łusiak, P., & Żukiewicz-Sobczak, W. (2023). Development of alternative protein sources in terms of a sustainable system. *Sustainability, **15*** (16), 12111.

Soni, R., Jain, N. K., Shah, V., Soni, J., Suthar, D., & Gohel, P. (2020). Development of probiotic yogurt: Effect of strain combination on nutritional, rheological, organoleptic and probiotic properties. *J. Food Sci. Technol,* **57**, 2038-2050.

Tournas, V., Stack, M. E., Mislivec, P. B., Koch, H. A. and Bandler, R. (2001). BAM: yeasts, molds and mycotoxins. *Bacteriological analytical manual,* **8**.

Vargas, M., Cháfer, M., Albors, A., Chiralt, A. and González-Martínez, C. (2008). Physicochemical and sensory characteristics of yoghurt produced from mixtures of cows' and goats' milk. *Int. Dairy J.* **18** (12), 1146-1152.

Vashishth, R., Semwal, A., Naika, M., Sharma, G. and Kumar, R. (2021). Influence of cooking methods on antinutritional factors, oligosaccharides and protein quality of underutilized legume Macrotyloma uniflorum. *J. Food Res. Int.* **143**, 110299.

Verma, A. and Mogra, R. (2013). Psyllium (Plantago ovata) husk: a wonder food for good health. *Int. J. Sci. Res.* **4** (9), 1581-1585.

Yang, M., Kwak, J. S., Jang, S., Jia, Y. and Park, I. (2013b). Antioxidant activity of soybean yogurt added tomato extract by Bacillus subtilis and Lactobacillus plantarum. *The Korean J. Food Nutr.* **26** (2), 280-286.

Yeo, S. K., & Liong, M. T. (2010). Effect of prebiotics on viability and growth characteristics of probiotics in soymilk. *J. Sci. Food. Agric.,* ***90***(2), 267-275.

Zhao, D. and Shah, N. P. (2014). Changes in antioxidant capacity, isoflavone profile, phenolic and vitamin contents in soymilk during extended fermentation. *J. Food Sci. Technol*. **58** (2), 454-462.